REMOTE OPTICAL PATH FOR CAPILLARY ELECTROPHORESIS INSTRUMENT

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to an apparatus for performing capillary electrophoresis and more specifically to the optical path for such an apparatus.

2. Description of the Prior Art

Capillary electrophoresis (CE) is a chemistry separation technique which utilizes the differences in solute electrophoretic velocity to isolate the various components of a sample. FIG. **1** depicts a typical CE apparatus. A high intensity electrical field supplied by high voltage power supply **10** is applied across a teflon, glass, or quartz separation capillary tube **12** of narrow inside diameter (5 to 400 micrometers) containing an electrolytic buffer solution. For an uncoated, open capillary tube, the presence of the electrical field imparts motion to charged and uncharged moieties present in the buffer through two mechanisms: electro-osmotic (endoosmotic) flow and electrophoretic force. Flow of buffer (or sample from sample vial **14**) through capillary **12** is detected by a detector **16**.

Electro-osmotic flow is the bulk flow of buffer from a first buffer vial **18** to a second buffer vial **19** through capillary **12** due to the shearing movement of a diffuse layer of cations past a more firmly held, dense layer, interacting with integral, anionic groups of the capillary wall. Factors which influence the velocity of electroosmotic flow are: electrical field strength; buffer dielectric constant; zeta potential (the electrical potential existing between diffuse and compact cationic layers); and buffer viscosity (which is dependent on bulk properties of the buffer and the temperature of the buffer). For electro-osmotically driven, packed capillary, reverse phase chromatography applications, solvents of use are any normally used solvent for standard reverse phase liquid chromatography.

Electrophoretic force is the force applied to charged particles residing in an electrical field, and neutral or uncharged molecules are not affected. Positively charged molecules (cations) migrate towards the cathode while negatively charged molecules (anions) move towards the anode. Factors controlling solute electrophoretic velocity are: molecular charge; electrical field strength; viscosity of the migration media; and solute molecular geometric factors.

The net velocity at which a solute travels in an uncoated, open capillary tube during CE is the vector sum of the electro-osmotic and electrophoretic velocities. Buffer viscosity plays a significant role for both of these phenomenon. Both electrophoretic and electro-osmotic velocities are inversely proportional to buffer viscosity, thus affecting the net migration velocity for all solutes.

When an electrical field is applied to a capillary which contains buffer, joule heating occurs. Accordingly the temperature of the buffer within the capillary increases until a steady state of heat exchange between the capillary and its surrounding environment is achieved. Consequently the ultimate buffer temperature is dependent upon the ambient temperature surrounding the capillary. Because of the temperature dependence of viscosity, the mobility of a solute in a given buffer within a given capillary in a given electrical field is largely determined by ambient temperature. For temperatures between 15° to 30° C., a 1° C. temperature increase results in an approximate 2 percent decrease in viscosity, increasing solute net velocity by 2 percent.

As is the case in many chromatographic techniques, solute identity is linked to migration time and velocity. For one form of CE known as capillary zone electrophoresis, samples are loaded into the capillary as a slug or plug. The latter may be achieved by application of an electrical field or some hydrodynamic force (vacuum or pressure head). An electrical field is then applied and the solutes migrate, as bands, down the capillary at their respective net velocities. Differences among these velocities create the primary mechanism for solute separation. These solute bands are then detected by monitoring a bulk property of the buffer such as refractive index, photometric absorbance, fluorescence, electrical conductivity, or thermal conductivity. The time period extending from the initiation of the separatory process to the point of solute detection is termed the migration time. The net velocity is determined using the migration time and the distance traveled by the solute.

Because of the high efficiencies achieved in capillary electrophoresis, it is not uncommon to see peak widths as narrow as two to three seconds. For complex solute matrices, multiple peaks may be separated by as little as two to three seconds in migration time. Consequently, a twenty minute CE run in which the temperature has changed by 0.1° C. can experience changes in migration time by as much as 2.4 seconds, possibly causing improper solute identification. Thus, efficient temperature regulation is required.

In the prior art, a capillary tube **12** as used in an electrophoresis instrument is supported in a variety of ways, depending on whether tube **12** is to be cooled by air, by liquid, or by metal plates in contact with the capillary tube. Cooling of tube **12** is important since the electrophoresis process subjects the capillary tube to a very high voltage which causes joule heating in the capillary tube. It is important to maintain the temperature of the tube at a stable predetermined temperature so as to be able to make measurements at a known temperature. Various schemes have been suggested for supporting and cooling the capillary tube, all of which have significant disadvantages and many of which are not suitable for air cooling purposes.

Prior art electrophoresis and similar spectrographic instruments typically include an optical path as shown in FIG. **2**, which includes two light sources **22, 24** each of which provides a different spectra. Typically one light source **22** is a deuterium (D_2) source and the second light source **24** is a tungsten (W) light source. A movable shutter **26** is provided in front of light sources **22, 24** so as to switch in light source **22** or light source **24** depending on which spectra is desired. A light beam **28** from either light source is then passed through baffles **29** onto a concave holographic grating **30** or similar diffraction device, and then is focused into beam splitter **32** through baffles **33**.

Beam splitter **32** in one form in the prior art is a short length of optical fibers. In the typical prior art instrument, a portion of the light transmitted to some of the optical fibers emerges from the beam splitter **32** at reference arm **34** and is sent via window **36** to a reference photodetector **38** which detects the reference light beam for purposes of comparison. The remainder of the light transmitted through beam splitter **32** is transmitted through a longer length of optical fibers to sample end **40** of the beam splitter and is focused using a lens **42** into